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Award Number: W81XWH-14-1-0569

TITLE: Validation and Interrogation of Differentially Expressed and Alternatively Spliced Genes in African-American Prostate Cancer

PRINCIPAL INVESTIGATOR: Steven Patierno

CONTRACTING ORGANIZATION: Duke University
Durham, NC 27708

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 September 2014 – 29 September 2015

4. TITLE AND SUBTITLE

Validation and Interrogation of Differentially Expressed and Alternatively Spliced Genes in African-American Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0569

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. These studies address the urgent need to elucidate the molecular mechanisms underlying the more aggressive prostate cancer biology in African American (AA) men. Specifically, our objectives are to 1) expand our sample cohort and delineate the relationship between genetic/epigenetic/post-transcriptional factors in AA prostate cancer and Gleason grade and 2) manipulate splicing using novel splice-switching oligonucleotides (SSOs) and determine functional outcomes. Toward these objectives, we have opened our GENomics of Cancer DisparitiEs Study to obtain AA and white prostate cancer blood and tissue specimens. For all tissue specimens collected, we have screened for tumor content, determined Gleason grade, isolated DNA and RNA and annotated. In addition, we have developed SSOs to manipulate *PIK3CD* alternative splicing, to correct aberrant splicing leading to production of AR-V7 and to drive production of inhibitory androgen receptor and epidermal growth factor receptor isoforms. Ultimately, this study will establish the genetic/epigenetic/post-transcriptional differences between AA and white prostate cancer and their relevance to tumor biology, which will pave the way toward identification of novel biomarkers and/or molecular targets for precision medicine that will reduce prostate cancer health disparities for AAs and improve outcomes for men of all races with aggressive disease.

15. SUBJECT TERMS

Prostate cancer, health disparities among racial groups, molecular mechanisms, differential gene expression, alternative RNA splicing, epigenetic alterations, clinical tumor aggressiveness

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

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INTRODUCTION: African American (AA) men exhibit 2-fold higher incidence and 3-fold higher mortality rates from prostate cancer compared to white men. Although much of this disparity remains after controlling for factors related to access to care, very few studies have utilized this population-based difference to identify molecular mechanisms of tumor aggressiveness. The studies proposed here address the urgent need to elucidate the molecular mechanisms underlying the more aggressive prostate cancer biology in AA men. Our objectives are to 1) expand our sample cohort and delineate the relationship between genetic/epigenetic/post-transcriptional factors in AA prostate cancer and Gleason grade and 2) manipulate splicing using novel splice-switching oligonucleotides and determine functional outcomes. Establishing the underlying genetic/ epigenetic/post-transcriptional differences between AA and white prostate cancer and the biologic relevance of these differences to tumor biology will identify novel precision biomarkers and/or molecular targets for precision medicine interventions that will have profound implications for the prevention, screening, diagnosis and management of prostate cancer in AA men as well as men of all races with aggressive disease. Specifically, if positive, these genetic/epigenetic/post-transcriptional differences could be developed as prognostic markers, in the context of Gleason grade and other prognostic variables, to delineate patients at greater risk of progressing on active surveillance or through localized therapy. In addition, the causal relationship of these pathways would help to rationalize specifically targeted therapy in selected patients.

KEYWORDS (20 words): Prostate cancer, health disparities among racial groups, molecular mechanisms, differential gene expression, alternative RNA splicing, epigenetic alterations, clinical tumor aggressiveness

ACCOMPLISHMENTS:

What were the major goals of the project?

Task 1. Validate differentially expressed and spliced candidate genes in AA prostate cancer in an expanded sample cohort and define the relationship between these genes and Gleason grade. Months 1-21. 15% complete (please see progress for Task 1 and changes/problems section).

Task 2. Define the biologic significance of differences in cis-acting splicing elements of alternatively spliced candidate genes in AA prostate cancer to alternative splicing events specific to AA prostate cancer and define the relationship between these patterns and Gleason grade. Months 21-36. Please see preliminary data for Task 2 and Tables 1, 2 and 3.

Task 3. Use splice-switching oligonucleotides to delineate the biologic relevance of race-related differentially spliced genes involved in PIK3CD and MET signaling. Months 1-24. 25% complete (please see progress and preliminary data for Task 3 and Figure 1).

What was accomplished under these goals?

Progress for Task 1: To obtain individual patient African American and white prostate cancer tissue specimens and patient-matched blood specimens, we have successfully obtained IRB approval and opened our GENomics of Cancer DisparitiEs (GENCADE) Study and accompanying database. For all tissue specimens collected to date, we have screened the specimens for tumor content, determined the Gleason grade, isolated cellular DNA and RNA and confirmed adequacy of yield and quality of these nucleic acids for downstream analyses and annotated the specimens. We have encountered an unanticipated slower rate of accrual in our first year collecting the specimens (please see changes/problems section).

Preliminary data for Task 2: While we obtain individual patient African American and white prostate cancer tissue specimens and patient-matched blood specimens, we have completed a preliminary analysis of publically available data in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) and the Multiethnic Cohort Study (MEC). This analysis has revealed that there are SNPs located in regulatory regions of the alternatively spliced genes we have previously shown contribute to the aggressive prostate cancer in African Americans that have the potential to affect splicing and that significantly associate with prostate cancer risk (please see Table 1), aggressiveness (please see Table 2) and survival (please see Table 3) in white and African American groups.

Progress and preliminary data for Task 3: We have successfully designed and synthesized chemically modified splice-switching oligonucleotides to manipulate *PIK3CD* alternative splicing. Splice-switching oligonucleotides targeting the exon 23 junction or a putative enhancer have been designed and synthesized to produce the *PIK3CD* short splice variant, found in AA prostate cancer, and a splice-switching oligonucleotide targeting a putative repressor has been designed and synthesized to produce the *PIK3CD* long splice variant, found in white prostate cancer. In addition, we have designed and synthesized novel chemically modified splice switching oligonucleotides to: 1) correct aberrant splicing leading to production of AR-V7, an androgen receptor variant that lacks the ligand binding domain, is constitutively active and associates with castrate resistant prostate cancer, poorer clinical outcomes and resistance to androgen ablation/androgen receptor inhibition therapies, 2) drive production of AR45, an androgen receptor variant that has a unique, non-functional transactivation domain and acts in a dominant-negative manner and 3) drive production of inhibitory or dominant-negative epidermal growth factor receptor isoforms, lacking the transmembrane or tyrosine kinase domain, respectively. Transfection of prostate cancer cells with the SSO to limit AR-V7 decreases AR-V7 RNA in a dose-dependent manner. Following transfection of prostate cancer cells with the SSO to produce AR45, an increase in AR45 RNA is seen as well as a simultaneous decrease in wild type androgen receptor RNA and in AR-V7 RNA is seen. In addition, a modulation of androgen receptor signaling is detected, with decreases in RNAs corresponding to a subset of androgen receptor-induced target genes and increases in RNAs corresponding to a subset of androgen receptor-repressed target genes being detected. Transfection of prostate cancer cells with SSOs to produce inhibitory or dominant-negative epidermal growth factor receptor isoforms increase RNA corresponding to these isoforms in a dose-dependent manner. Furthermore, a decrease in phosphorylated epidermal growth factor receptor protein is detected (please see Figure 1).

Along with our qualified collaborator and his laboratory, we are preparing a manuscript focusing on our identification of a large number of alternative RNA splicing events in cancer-associated pathways in white and African American prostate cancer, with a subset of these events also being detected in patient-matched normal prostate specimens. The events have biological significance, with one isoform of *PIK3CD* versus the alternative associating with increased oncogenic signaling, proliferative and invasive phenotypes. In addition, the events have clinical significance as biomarkers and molecular targets for therapeutics, with one *PIK3CD* isoform versus the alternative associating with sensitivity or resistance to small molecule inhibition of the protein encoded by the alternatively spliced gene. The aforementioned biological significance was determined using isoform specific knockdowns with siRNAs. As mentioned earlier regarding our progress and preliminary data for Task 3, we have successfully designed and synthesized chemically modified splice-switching oligonucleotides to manipulate alternative splicing of this target and will be testing the effects of these splice-switching oligonucleotides on prostate cancer cell biology as well as therapeutic efficacy in prostate cancer xenografts and prostate cancer patient-derived explant models.

Table 1. Significant associations between splicing-related SNPs in prioritized target genes and prostate cancer ***risk*** in either PLCO European population or MEC African American population.

		PLCO European population(N=2251)				MEC African American(N=1371)			
SNP	Gene	A1_ Cases[1]	A1_ Controls[1]	OR(95%CI)[2]	*P*[2]	A1_ cases[1]	A1_ controls[1]	OR(95%CI)[2]	*P*[2]
rs11651 (G/A)	*FN1*	0.35	0.32	1.19(1.01-1.38)	0.033	0.11	0.11	0.97(0.75-1.25)	0.819
rs13652 (C/T)	*FN1*	0.13	0.11	1.29(1.02-1.63)	0.031	0.19	0.19	0.99(0.82-1.21)	0.949
rs36104025 (G/C)	*COL6A3*	0.09	0.08	1.34(1.02-1.75)	0.036	--	--	--	--
rs3790993 (C/G)	*COL6A3*	0.45	0.46	0.85(0.73-0.99)	0.036	0.43	0.44	0.96(0.82-1.12)	0.627
rs1058425 (T/C)	*SEMA3C*	0.1791	0.1962	0.85 (0.7-1.03)	0.094	0.3982	0.4336	0.82 (0.70-0.96)	0.0145
rs1714987 (G/C)	*ACACA*	0.17	0.2	0.8(0.66-0.96)	0.02	0.27	0.27	0.98(0.82-1.17)	0.823
rs59638227 (G/A)	*FASN*	0.30	0.29	1.02 (0.86-1.21)	0.792	0.1154	0.1425	0.74 (0.58-0.94)	0.0142

[1] Frequency of A1 allele.
[2] Adjusted for the top three principle components and age at diagnosis.

Table 2. Significant associations between splicing-related SNPs in prioritized target genes and prostate cancer ***aggressiveness*** in either PLCO European population or African American population.

		PLCO population				African American			
SNP	Gene	A1_ Aggressive (N=237)[1]	A1_Non Aggressive (N=843)[1]	OR(95%CI)[2]	*P*[2]	A1_ Aggressive (N=234)[1]	A1_Non-Aggressive (N=436)[1]	OR(95%CI)[2]	*P*[2]
rs1714987 (G/C)	*ACACA*	0.134	0.182	0.70(0.52-0.93)	0.015	0.260	0.271	0.97(0.73-1.28)	0.826

rs17275986 (G/A)	*SEMA3C*	0.213	0.212	0.98(0.76-1.25)	0.861	0.061	0.096	0.59(0.36-0.96)	0.034
rs362708 (G/A)	*RELN*	NA	NA	NA	NA	0.414	0.354	1.30(1.00-1.67)	0.047
rs3817552 (G/C)	*MYBPC1*	0.148	0.144	1.05(0.78-1.41)	0.7373	0.144	0.099	1.53(1.06-2.22)	0.024
rs8546 (A/G)	*NCOR2*	0.159	0.160	0.98(0.74-1.31)	0.9047	0.133	0.092	1.49(1.01-2.22)	0.046
rs15736 (G/A)	*WDR4*	0.359	0.381	0.90(0.73-1.11)	0.3163	0.442	0.368	1.40(1.09-1.80)	0.009
rs11911090 (T/C)	*WDR4*	0.101	0.081	1.27(0.90-1.78)	0.174	0.185	0.141	1.40(1.01-1.95)	0.044
rs2248490 (C/G)	*WDR4*	0.491	0.492	1.00(0.82-1.23)	0.9697	0.356	0.276	1.51(1.16-1.99)	0.003

[1] Frequency of A1 allele.
[2] Adjusted for the top three principle components and age at diagnosis.

Table 3. Significant associations between splicing-related SNPs in prioritized target genes and ***survival*** of 1150 prostate cancer patients in PLCO.

		Overall (N = 1150)			Aggressiveness[2] (N =237)		Non-aggressiveness (N =843)	
SNP	Gene	A1A1/A1A2/A2A2	HR (95% CI)[1]	*P*[1]	HR (95% CI)[1]	*P*[1]	HR (95% CI)[1]	*P*[1]
rs3738073 (T/C)	*RHOU*	48/378/723	1.34 (1.06-1.68)	0.013	1.65 (1.02-2.65)	0.04	1.33 (1.00-1.77)	0.047
rs7596677 (T/A)	*FN1*	66/395/647	1.07 (0.85-1.34)	0.564	1.59 (1.01-2.50)	0.045	0.92 (0.69-1.23)	0.577
rs1131296 (A/G)	*COL6A3*	185/572/394	0.88 (0.71-1.08)	0.21	0.64 (0.42-0.99)	0.044	0.95 (0.74-1.22)	0.69
rs1880959 (A/C)	*SEMA3C*	8/215/921	0.71 (0.5-1.02)	0.067	0.82 (0.41-1.65)	0.575	0.63 (0.40-0.99)	0.047
rs2229862 (A/G)	*RELN*	3/118/1030	1.53 (1.04-2.26)	0.031	3.56 (1.71-7.43)	0.001	1.02 (0.61-1.72)	0.933
rs362691 (C/G)	*RELN*	11/236/901	0.85 (0.62-1.17)	0.328	0.38 (0.17-0.87)	0.022	1.03 (0.71-1.49)	0.888
rs9666607 (A/G)	*CD44*	109/493/549	1.28 (1.04-1.58)	0.019	1.13 (0.75-1.70)	0.558	1.29 (1.00-1.67)	0.050
rs1467558 (T/C)	*CD44*	45/337/769	1.24 (0.97-1.57)	0.081	1.10 (0.67-1.80)	0.701	1.37 (1.02-1.82)	0.034
rs17706535 (G/A)	*LMO7*	8/216/903	0.91 (0.65-1.27)	0.573	0.47 (0.23-0.96)	0.039	1.09 (0.72-1.64)	0.684
rs15736 (A/G)	*WDR4*	167/540/444	1.25 (1.03-1.52)	0.023	1.32 (0.90-1.94)	0.157	1.15 (0.90-1.46)	0.259

[1] Adjusted for age, stage, gleason score, primary treatment and the top three principle components.
[2]Aggressive was defined as "stage III/IV or gleason score ≥ 8".

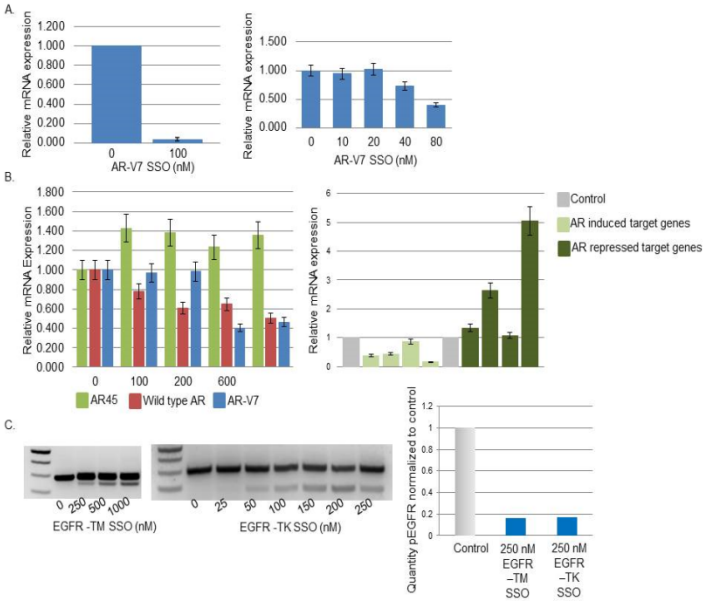

Figure 1. A. Dose-dependent inhibition of AR-V7 by the AR-V7 SSO. B. The AR45 SSO drives production of AR45 simultaneously decreasing wild type AR and AR-V7 and modulating AR signaling. C. EGFR-TM and -TK SSOs drive production of dominant-negative EGFR variants in a dose-dependent manner and inhibit pEGFR expression.

What opportunities for training and professional development has the project provided?

Training and professional development has been provided for Jennifer A. Freedman, PhD, Co-investigator and Bonnie LaCroix, Research Analyst I. Dr. Freedman has expanded her expertise in performing all aspects of translational research by writing the GENCADE IRB protocol, designing the GENCADE REDCaP database, creating the GENCADE IRB-approved patient handout and collaborating with members of the Genitourinary Oncology clinical research team to implement the GENCADE Study. In addition, this project has given her an opportunity to design additional splice-switching oligonucleotides and continue to increase her knowledge regarding prostate cancer health disparities among racial groups. Furthermore, this project has provided an opportunity for Dr. Freedman to further develop her skills in scientific management and mentoring of members of the Genitourinary Oncology Laboratory, specifically the Research Analyst I working on this project. Finally, Dr. Freedman attended and presented this study at a poster session at the AACR Cancer Health Disparities

Conference this past November. Mrs. LaCroix has expanded her technical molecular biology expertise and her knowledge of prostate cancer health disparities among racial groups.

How were the results disseminated to communities of interest?

Outreach activities have been undertaken to increase participation, including minority participation, in our GENCADE Study. In collaboration with the Duke Cancer Institute's Office of Health Equity and Disparities, we have approached patients participating in our annual community Men's Health Fair and have designed, produced and implemented use of our GENCADE IRB-approved patient handout.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we expect to near completion of our collection of individual patient African American and white prostate cancer tissue specimens and patient-matched blood specimens with accompanying annotation and DNA and RNA isolation. Once complete, we will be performing ancestral genotyping using DNA isolated from the blood specimens and we will be interrogating gene expression, alternative splicing and variations in *cis*-acting splicing elements using DNA and RNA from the tissue specimens. In addition, we will be transfecting prostate cancer cell lines derived from African American and white patients with our splice-switching oligonucleotides targeting alternative splicing of *PIK3CD* to assess resulting levels of splice variants and effects on tumor cell biology. Furthermore, we will be designing, synthesizing and assessing efficacy of splice-switching oligonucleotides targeting alternative splicing of *MET*. Finally, we will be transfecting prostate cancer cell lines derived from AA and white patients, expressing appropriate levels of AR or EGFR, with the AR-V7, AR45, EGFR –TM or EGFR -TK SSO and assessing the resulting alterations in downstream target expression, transactivation activity, proliferation (for AR studies, androgen-dependent and –independent), migration, invasion, colony formation, apoptosis and sensitivity to inhibitors (for AR studies, enzalutamide, and for EGFR studies, tyrosine kinase inhibitors).

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Collection of individual patient African American and white prostate cancer tissue specimens and patient-matched blood specimens will contribute to the goal of accumulating racially diverse preclinical prostate tumor models to assess the biologic significance of the factors contributing to the clinical aggressiveness of African American prostate cancer. In addition, development of splice-switching oligonucleotides to modulate alternative splicing of *PIK3CD*, to correct aberrant splicing leading to production of AR-V7 and to drive production of inhibitory androgen receptor and epidermal growth factor receptor isoforms will further our understanding of the contribution of this molecular mechanisms to African American prostate cancer and have the potential to yield novel therapeutic modalities to combat prostate cancer in African American men as well as men of all races with aggressive disease driven by these mechanisms.

What was the impact on other disciplines?

Similar correlations between specific splice variants and aggressiveness have been identified in a wide range of cancers. Therefore, the splice-switching oligonucleotide technology being developed here has the potential to have broader applicability.

What was the impact on technology transfer?

Along with our qualified collaborator, a US Patent Application has been filed regarding alternative splicing variants of genes associated with prostate cancer risk and survival (US 2014/0364483 A1). The splice-switching oligonucleotides targeting the oncogenic androgen receptor and epidermal growth factor receptor RNA isoforms are the subject of a pending patent application at Duke University Medical Center.

What was the impact on society beyond science and technology?

The outreach activities we have undertaken to increase participation, including minority participation, in our GENCADE Study and our physician-patient informed decision-making and informed consent process in our GENCADE Study will improve public knowledge regarding prostate cancer, prostate cancer health disparities among racial groups and clinical research.

CHANGES/PROBLEMS:

Changes in approach and reasons for change.

In order to increase collection of individual patient African American and white prostate cancer tissue specimens, in addition to collecting cores from transrectal ultrasound biopsies of the prostate, we have also started to collect cores from magnetic resonance fusion biopsies of the prostate, 3D transperineal mapping biopsies of the prostate and from the prostate after it has been resected.

Actual or anticipated problems or delays and actions or plans to resolve them.

We have encountered an unanticipated slower rate of accrual in our first year collecting individual patient African American and white prostate cancer tissue specimens and patient-matched blood specimens. This has been a result of the record, extremely high volume of patients our Urology clinic has been seeing of late. We have recognized the need to optimize our approach to more seamlessly integrate into these pressured clinical operations, allowing us to navigate within this environment while not interfering with their need to maximize patient flow. To optimize our approach, we have made several changes. Regarding personnel, we have obtained the support of the Chief of the Division of Urologic Surgery, initiated regular meetings with the urologic surgeons and engaged the nursing staff to incorporate notification of the GENCADE Study into the real-time clinic workflow. In addition, we have designed, produced and implemented use of an IRB-approved GENCADE patient handout, which is given to patients in the context of physician-patient informed decision-making and we have obtained IRB approval to follow up with patients receiving the handout via telephone to obtain informed consent. Since raising awareness and implementing all of the aforementioned changes, we have already seen an increase in our rate of accrual and anticipate being able to complete collection of individual patient African American and white prostate cancer tissue specimens and patient-matched blood specimens during the next reporting period.

Changes that had a significant impact on expenditures.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

PRODUCTS:

Publications, conference papers, and presentations.

Nothing to report.

Website(s) or other Internet site(s).

Nothing to report.

Technologies or techniques.

Splice-switching oligonucleotides to manipulate *PIK3CD* alternative splicing, to correct aberrant splicing leading to production of AR-V7 and to drive production of inhibitory androgen receptor and epidermal growth factor receptor isoforms have been developed and, once complete, we plan to submit this research data for publication making our scientific discovery open to the scientific community.

Inventions, patent applications, and/or licenses.

Along with our qualified collaborator, a US Patent Application has been filed regarding alternative splicing variants of genes associated with prostate cancer risk and survival (US 2014/0364483 A1). The splice-switching oligonucleotides targeting the oncogenic androgen receptor and epidermal growth factor receptor RNA isoforms are the subject of a pending patent application at Duke University Medical Center.

Other products.

Nothing to report.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	**Steven Patierno, PhD, PI**
Project Role:	Principal Investigator

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	Provided senior level oversight and direction, contributed to implementation of the GENCADE study
Funding Support	P30CA014236-42 See attached other support/ DOD

Name:	**Daniel J George, MD**
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	Contributed to implementation of the GENCADE study, contributed to oversight and direction
Funding Support	See attached other support/ DOD

Name:	**Susan K Murphy,PhD**
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	Planned pilot experiments to generate epigenetic data using prostate cancer cell lines derived from African American and white patients and GENCADE specimens collected to date
Funding Support	See attached other support/ DOD

Name:	**Jennifer A Freedman, PhD**
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.1
Contribution to Project:	Wrote GENCADE IRB protocol, designed GENCADE REDCaP database, created GENCADE IRB-approved patient handout and contributed to implementation of the GENCADE study in collaboration with the Genitourinary Oncology clinical research team, designed splice-switching oligonucleotides to manipulate PIK3CD alternative splicing, managed and mentored Research Analyst I, Bonnie LaCroix
Funding Support	See attached other support/ DOD

Name:	**Jason A Somarelli**

Project Role:	Postdoctoral Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	No contribution this reporting period
Funding Support	Prostate Cancer Foundation Award

Name:	**Yuan Wu**
Project Role:	Biostatitician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	No contribution this reporting period
Funding Support	

Name:	**Zhiqing Huang**
Project Role:	Senior Research Scientitist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	No contribution this reporting period
Funding Support	

Name:	**Carole Grenier**
Project Role:	Research Analyst I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	No contribution this reporting period
Funding Support	

Name:	**Norman Lee, PhD**
Project Role:	GWU PI
Researcher Identifier (e.g. ORCID ID):	NHLEE1
Nearest person month worked:	1.2
Contribution to Project:	Coordinated efforts between Duke and GWU. Was responsible for experimental design of primer pairs and oversaw all laboratory activities
Funding Support	See attached other support/ DOD

Name:	**Bi-Dar Wang, PhD**
Project Role:	GWU Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	Performed RTPCR testing and validation of primer pairs for amplification of short and long isoforms

	of PI3KCD in AA and EA PCa cell lines and patient specimens
Funding Support	See attached other support/ DOD

Name:	**Ramez Andrawls, MD**
Project Role:	GWU Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	No change
Contribution to Project:	Provided prostate cancer specimens for testing of primer pairs
Funding Support	See attached other support/ DOD

Collaborating Organization – George Washington University Medical Center

RT-PCR validation of L and S variants of PIK3CD in PCa cell lines and patient specimens. The Lee lab has validated a series of primer pairs for the amplification of the short (S) and long (L) isoforms of PIK3CD in a panel of AA (MDA PCa 2b, E006AA) and EA PCa cell lines (PC-3, LNCaP and VCaP) as well as validation in prostate cancer specimens of AA or EA origin. Primer pairs for exons 20 through 24 consistently amplify 547bp (L form) and 379bp products (S form missing exon 23); and primer pairs for exons 20 through 25 consistently amplify 685bp (L form) and 517bp products (S). S isoform is consistently more highly expressed in AA cell lines, and L isoform is consistently more highly expressed in EA lines. Similar results are seen in patient specimens. Having validated these primer pairs, the next step will be to use these primer pairs to assess the ability of splice switching oligos (SSO), being developed by our Duke collaborators, to switch isoform usage in PCa cell lines and to test the functional consequences of isoform switching. Lastly, patient consent form in accordance to Duke protocols/procedures is being reviewed and finalized by GWU IRB.

RT-PCR validation of the long and short splice variants of PIK3CD is in AA and EA PCa cell lines. To further evaluate the functional role(s) of the splice variants of PIK3CD *in vitro*, we first applied RT-PCR assays to investigate whether the long and short splice variants of PIK3CD were expressed in PCa cell line models. Four commercially available PCa cell lines, including PC-3, LNCaP, VCaP and MDA PCa 2b (ATCC, Manassas, VA) were used in this RT-PCR validation experiment. PC-3, VCaP were derived from EA PCa patients with bone metastasis, and LNCaP was derived from EA patients with lymph node metastasis, while MDA PCa 2b was derived from AA patient with bone metastasis. RNA samples were purified from the EA and AA PCa cell lines using Qiagen RNeasy mini kit (Qiagen, Valencia, CA) and were subjected to the RT-PCR assays. RT-PCR results showed that the short variant of PIK3CD was enriched in AA PCa cell line MDA PCa 2b, whereas the EA cell lines (PC-3, LNCaP and VCaP) exhibited higher expression of PIK3CD long variant (**Figure 1a**). To further quantify the expression level of long and short variants of PIK3CD in the EA and AA PCa cell lines, EA cell line VCaP and AA cell line MDA PCa 2b (both derived from bone-metastasis) were selected to compare their expression levels of PIK3CD-L and –S variants. Forward primer on exon 20 and reverse primers on exon 24 and 25 were designed for the RT-PCR validation of the long and short splice variants of PIK3CD. Using the primer pair targeting exon 20 and 25, the long variant (685bp) and short variant (517bp, exon 23 is missing) were amplified by RT-PCR reactions. Quantification analysis showed that the ratios of PIK3CD-S/PIK3CD-L were 0.33 and 9.01 in VCaP and MDA PCa 2b, respectively (**Figure 1b, left panel**). By using another primer pairs for amplifying exon 20-24 transcripts, our RT-PCR results again showed

a higher PIK3CD-S (379bp)/PIK3CD-L (547bp) ratio in MDA PCa 2b (ratio of 3.02) compared to VCaP (ratio of 0.80) (**Figure 1b, right panel**). These results were consistent with our RT-PCR results in AA and EA PCa clinical samples, confirming that higher level of PIK3CD short variant is expressed in AA PCa compared to EA PCa.

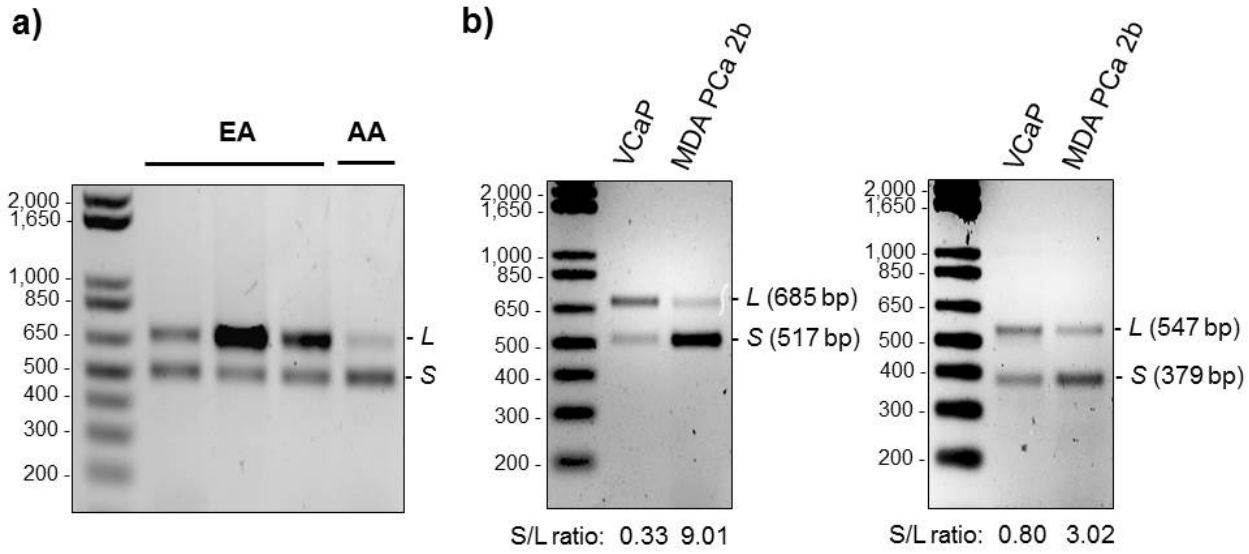

Figure 1. RT-PCR validation of PIK3CD splice variants in EA and AA PCa cell lines. **(a)** RNA samples purified from commercially available EA PCa cell lines (PC-3, LNCaP and VCaP; lane 1-3) and AA PCa cell line (MDA PCa 2b, lane 4) were used in the RT-PCR assays. EA cell lines expressed both long and short variants (long variant as major isoform) and AA cell line has higher expression of PIK3CD short variant. The primers targeting exon 20 and 24 were used in the PCR reactions. **(b)** Quantification analysis of long and short variants of PIK3CD in EA cell line VCaP and AA cell line MDA PCa 2b. Primers for exon 20 and 25 were used to amplify the long (685bp) and short (517bp) variants of PIK3CD (left panel). Primers for exon 20 and 24 were used to amplify the long (547bp) and short (379bp) variants of PIK3CD (left panel). The S/L ratio was determined by measuring the gel images of PIK3CD-S and –L transcripts using Image J program.

References:

1. Gibas Z, Becher R, Kawinski E, Horoszewicz J, Sandberg AA. A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet Cytogenet 1984;11: 399-404.

2. Navone NM, Olive M, Ozen M*, et al.* Establishment of two human prostate cancer cell lines derived from a single bone metastasis. Clin Cancer Res 1997;3: 2493-500.

OTHER SUPPORT

Steven Patierno, Ph.D.

ACTIVE

5P30-CA14236-41 (Kastan, M, PI) 04/01/15 – 12/31/19 10%
National Institutes of Health $253,125
Nick Mitrano, National Institute of Health/NCI
6120 Executive Blvd, EPS Suite 243, MSC 7150
Bethesda, MD 208792
Senior Leaders- Duke Comprehensive Cancer Core Grant
Goal: Make preeminent contributions to understanding, preventing, detecting, diagnosing and treating cancer through laboratory investigation, clinical research, cancer prevention and control, research, patient care, education and interaction with individuals and organizations outside the University.

W81XWH-14-1-0569 (Patierno, PI) 09/30/2014 - 09/01/2017 1%
Department of Defense $595,633
Kathy E. Robinson, Contracting Officer
DFAS-INDY VP GFEBS
8899 E 56th Street
Indianapolis IN 46249-3800
Validation and interrogation of differentially expressed and alternatively spliced genes in Africanc American prostate cancer.
The specific aims of this proposed research are 1) to validate differentially expressed and spliced candidate genes in AA prostate cancer in an expanded sample cohort and define the relationship between these genes and Gleason grade, 2) to define the biologic significance of differences in cis-acting splicing sequences in primary transcripts of alternatively spliced candidate genes in AA prostate cancer to alternative splicing events specific to AA prostate cancer and define the relationship between these patterns and Gleason grade, and 3) to use splice-switching oligonucleotides to delineate the biologic relevance of race-related differentially expressed and spliced genes involved in PIK3CD and MET signaling

Overlap: None

SPOC approved by Gloria Bass, DCI – 10/12/15

Other Support

FREEDMAN, JENNIFER A.

ACTIVE

W81XWH-14-1-0569 (Patierno, PI)	09/30/2014 - 09/01/2017	10%
Department of Defense		$595,633
Kathy E. Robinson, Contracting Officer		
DFAS-INDY VP GFEBS		
8899 E 56th Street		
Indianapolis IN 46249-3800		

Validation and interrogation of differentially expressed and alternatively spliced genes in Africanc American prostate cancer.

The specific aims of this proposed research are 1) to validate differentially expressed and spliced candidate genes in AA prostate cancer in an expanded sample cohort and define the relationship between these genes and Gleason grade, 2) to define the biologic significance of differences in cis-acting splicing sequences in primary transcripts of alternatively spliced candidate genes in AA prostate cancer to alternative splicing events specific to AA prostate cancer and define the relationship between these patterns and Gleason grade, and 3) to use splice-switching oligonucleotides to delineate the biologic relevance of race-related differentially expressed and spliced genes involved in PIK3CD and MET signaling

OVERLAP

NONE

Spoc approved Gloria Bass, 10.25.15

Other Support

GEORGE, DANIEL

*Changes are indicated with an * before the title.*

The following are no longer active:

W81XWH-10-1-0339
UCSF: subcontract: Predictive Markers in RCC
AstraZeneca trial ZD4054
Cougar Biotechnology trial CB7630
Genentech trial GDC-0980
Novartis trials CRAD001C2472, CLBH589C2205, CTK1258A2116
Pfizer trial 2005-0136
Wyeth Pharmaceuticals trial 3066K1-3311-WW
Cancer and Leukemia Group B Foundation protocol 90206
UCSF trial 5516sc
Beth Israel Deaconess Medical Center trial

ACTIVE

Clinical Consortium - Clinical Research Site
20% effort
Department of Defense (W81XWH-14-2-0179)
Pamela L. Fisher
USA Medical Research ACQ
820 Chandler Street, Ft. Detrick, MD 21702
09/30/14 – 9/29/17
$199,365 (current year direct dollars)
Goal/Specific Aim(s): The goals/aims/tasks of this project include the following:

1. To maintain the progress made since the initial PCRP CCA in January 2007 and continue current growth velocity.
2. Will maintain operational compliance with CDMRP and Consortium procedures.
3. Contribute to the consortium structure and processes.
4. Participate in consortium data collection and capture systems.

Novel Immune Modulating Cellular Vaccine for Prostate Cancer Immunotherapy
10% effort
Department of Defense (W81XWH-13-1-0423)
Pamela L. Fisher
USA Medical Research ACQ
820 Chandler Street
Ft. Detrick, MD 21702
9/30/13 - 9/29/16
$1,002,895
Goal/Specific Aim(s): Our overall goal is to design a prostate cancer immunotherapy strategy that will effectively: 1. Enhance the function of tumor antigen-specific T cells by targeted modulation of immune receptor function and 2. Lead to the development of a clinically effective prostate cancer immunotherapy, without inducing severe autoimmunity.

Validation and interrogation of differentially expressed and alternatively spliced genes in African American prostate cancer
1% effort
Department of Defense (W81XWH-14-1-0569)
Pamela L. Fisher
USA Medical Research ACQ
820 Chandler Street
Ft. Detrick, MD 21702
9/30/14 – 9/29/17
$595,633
Goal/Specific Aim(s): Our proposed research focuses on further understanding the biological differences between African American and Caucasian American prostate cancer. The specific aims of this proposed research are 1) to validate differentially expressed and spliced candidate genes in AA prostate cancer in an expanded sample cohort and define the relationship between these genes and Gleason grade, 2) to define the biologic significance of differences in cis-acting splicing sequences in primary transcripts of alternatively spliced candidate genes in AA prostate cancer to alternative splicing events specific to AA prostate cancer and define the relationship between these patterns and Gleason grade, and 3) to use splice-switching oligonucleotides to delineate the biologic relevance of race-related differentially expressed and spliced genes involved in PIK3CD and MET signaling.

*Development of Circulating Molecular Predictors of Chemotherapy and Novel Hormonal Therapy Benefit in Men with Metastatic Castration Resistant Prostate Cancer (mCRPC)
1% effort
Prostate Cancer Foundation
Helen Hsieh, VP, Finance and Administration
1250 Fourth Street
Santa Monica, CA 90401
8/1/14 – 8/1/16
$1,170,797 ($342,930 is a subcontract to WCMCU)
Goal/Specific Aim(s): Our long term goal is the development of predictive biomarkers of abiraterone, enzalutamide, and taxane therapy and the identification of patients most likely to benefit but also those men with mCRPC who should be offered combination or alternative therapies based on pre-treatment or treatment-emergent resistance. Specific Aim 1. Assessment of a CRPC molecular taxonomy based on circulating tumor cells (CTCs) in men unexposed to abiraterone acetate or enzalutamide therapy. Specific Aim 2. To describe treatment-emergent CRPC genotypes during abiraterone acetate, enzalutamide, and taxane-based systemic therapy using CTC and circulating biomarkers.

*Sub project Title – Oncolytic poliovirus immunotherapy for prostate cancer
1.5% effort
National Institutes of Health (5UL1TR001117-03)
Judith Musgrave
6701 Democracy Blvd,
BG 1DEM Room 1060
Bethesda, MD 20817

5/1/15 – 4/30/16
$100,000 (sub project award)
Goal/Specific Aim(s): The proposed preclinical studies will evaluate the ability of regional oncolytic poliovirus therapy to induce systemic anti-tumor immune responses and establish biomarkers of an effective immune response.

INDUSTRY SPONSORED RESEARCH **Aggregated Effort** **25.0% Effort**

Duke University lists aggregated effort assigned to the following eligible industry-sponsored clinical trial projects. Each of these individual projects has a varying need of effort depending on the type of activity currently in progress such as protocol development, start-up, patient recruitment, enrollment, follow-up, monitoring, data analysis, publication, and closeout. Faculty determines each project's need and adjust their effort between projects within the total aggregated effort assigned to the clinical projects.

*ACE-ST-005
Acerta Pharma (ACRTA-01)
1509 Industrial Road
San Carlos, CA 94070
6/10/15 – 8/19/17
$2,467,197
Goal/Specific Aim(s): This is a randomized Phase 2 trial of ACP-196 and Pembrolizumab Immunotherapy Dual CHECKpoint Inhibition in Platinum Resistant Metastatic Urothelial Carcinoma (RAPID CHECK study).

Evaluate the Risk of Seizure Among Subjects with Metastatic Castration-Resistant Prostate Cancer (mCRPC)
Astellas Pharma Global Development (9785-CL-0403)
ATTN: Research & Development
Northbrook, Illinois
4/1/12 - 3/31/17
$97,757
Goal/Specific Aim(s): This is a multicenter, single-arm, open-label, post-marketing safety study to evaluate the risk of seizure among subjects with metastatic castration-resistant prostate cancer (mCRPC) treated with enzalutamide who are at potential increased risk of seizure.

A Randomized, Blinded, Phase 2 Dose-Ranging Study of BMS-936558
Briston-Myers Squibb Company
345 Park Avenue
New York, NY 10154
7/1/11 – 6/30/19
$302,495
Goal/Specific Aim(s): The information gained from this pharmacogenetic research studies is expected to result in safer and more effective therapies and to lead to the discovery of new diagnostics, prognostics and molecular targets and pathways. To learn more about the association between a subject's genetic makeup and drug response, subjects will be asked to voluntarily give a blood sample so that scientists can perform exploratory

pharmacogenetic research.

A randomized discontinued study of XL184 in subjects with advanced solid tumors
Exelixis, Inc
Peter Lamb, PhD
170 Harbor Way, PO Box 511
South San Francisco, CA 94083
03/02/11 - 03/01/16
$235,782 (current year direct dollars)
Goal/Specific Aim(s): To evaluate the safety and tolerability of XL184 I subjects with advanced solid tumors, to correlate the pathway dysfunction of disease related genes or proteins and to further characterize the pharmacokinetic (pK) and pharmacodynamics parameters of XL184.

A randomized, double-blind, placebo-controlled phase II study to evaluate the efficacy and safety of pazopanib as adjuvant therapy for subjects with localized or locally advanced RCC following nephrectomy
GlaxoSmithKline
Peter T.C. Ho, M.D., PhD
5 Moore Drive
RTP, NC 27709
12/20/10 - 4/16/19
$293,734 (current year direct dollars)
Goal/Specific Aim(s): To evaluate disease free survival (DFS), overall survival (S), rate at end of each year, safety and quality of life.

A Phase 1/2 Open-Label, Multiple-Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of VT-464 in Chemotherapy-Naive Patients with Castration-Refractory Prostate Cancer
Innocrin Pharmaceutical / Viamet Pharmaceuticals
Attention: CEO
2250 Perimeter Park Drive
Suite 320
Morrisville, NC 27560
11/01/2013 - 10/31/2018
$220,415
Goal/Specific Aim(s): The primary objective of the study is to determine the safety and tolerability of orally-administered VT-464 in chemotherapy-na<symbol>239</symbol>ve patients with castration-refractory prostate cancer (CRPC).

ABI-RACE
Janssen Pharmaceutical, Inc.
Loye Rose
1125 Trenton-Harbourton Road
Titusville, NJ 08560-0200
06/01/2013 – 05/31/2018
$583,819

Goal/Specific Aim(s): Primary Objective – The primary goal is to prospectively estimate the median PFS of African American and Caucasian men with mCRPC to abiraterone acetate and prednisone. Secondary Objectives – PSA kinetics: to determine the duration of PSA response, time to nadir, and percent of men who achieve a PSA < 0.1 Radiographic assessments: to estimate the rate of objective response and incidence of bone flares Safety (NCI CTC v4.0) and tolerability, particularly incidence and grade of hypertension in the two populations. Exploratory Objectives – Describe the baseline profile of serum hormone levels (including testosterone, DHT, DHEA, DHEAS, estradiol), the change in levels with subsequent therapy (4 weeks), and their correlation with response to abiraterone acetate. Describe the germline SNP profiles of target genes involved in androgen signaling and target genes that have been shown to be differentially expressed in African American versus Caucasian American prostate cancers as well as a genome wide analysis (GWAS) in both African American and Caucasian men with mCRPC and their associations with response to abiraterone acetate

Axitinib (AG-013736) as Second Line Therapy for Metastatic Renal Cell Cancer; Axis Trial
Pfizer, Inc. (A4061032)
Martin Mackay
235 East 42nd Street
New York, NY 10017
11/12/08 – 9/1/19
$79,276 (current year direct dollars)
Goal/Specific Aim(s): A 2-arm, randomized, open-label, multi-center Phase 3 study of AG-013736 (axitinib) vs. sorafenib in patients with mRCC following failure of one prior systemic first-line regimen containing one or more of the following: sunitinib, bevacizumab + IFN α, temsirolimus, or cytokine(s).

A randomized double-blind phase 3 study of adjuvant sunitinib vs. placebo
Pfizer, Inc. (A6181109)
Martin Mackay
235 East 42nd Street
New York, NY 10017
$160,482
Goal/Specific Aim(s):The pPrimary objective of this project is: To demonstrate an improvement in disease-free survival (DFS) in high risk (per modified UISS criteria) subjects with RCC randomly assigned to adjuvant sunitinib 50 mg schedule 4/2: 4 weeks on, 2 weeks off for 1 year (Arm A), vs. Placebo (Arm B) after nephrectomy.

Ganetespib (STA-9090) in Combination with Carboplatin for A Patient with Metastatic Prostatic Ductal Carcinoma
Synta Pharmaceuticals Corp.
Safi Bahcall
45 Hartwell Avenue
Lexington, MA 02421
1/6/13 – 1/31/18
$0 (No funding provided)

Goal/Specific Aim(s): The goal of this project is to use Ganetespib (STA-9090) in Combination with Carboplatin for A Patient with Metastatic Prostatic Ductal Carcinoma. No financial support is being received from Synta Pharmaceuticals – only drug.

OVERLAP

No financial or scientific overlap

Prepared by Erin Dillard 10/28/15
Certified by

OTHER SUPPORT

Murphy, Susan K.
In the past year, Dr. Murphy has effort end on 5R01-DK085173-04, 5R01-ES016772-05, 5R21-AG041048-021R03CA171617-01, and OC100276 W81XWH-11-1-0469. She is now providing effort on W81XWH-14-0309 OC130493, R01-ES022216, and R01-ES016099

ACTIVE

Title: Epigenomic Consequences of Early Life Environmental Tobacco Smoke Exposure NICHES-Project 3 (Murphy)
Time Commitment: 10.6% 1.27 calendar
Supporting Agency: NIH/NIEHS 5P01-ES022831-03
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Grants Management Specialist: Wanda G Boggs Email: boggsw@niehs.nih.gov Phone: 919-316-4638
Performance Period: 06/05/2013 – 05/31/2018
Level of Funding: $226,892
Goals: The results will for the first time mechanistically link tobacco smoke exposure in early life to neurobehavioral aspects of ADHD through epigenetics. The identified genes are highly likely to pave the way for development of novel diagnostic, prognostic and therapeutic tools that will ultimately improve children's health by allowing for earlier recognition of risk and intervention opportunities.
Specific Aims: 1) Identify ETS-related methylation targets. We will test the hypothesis that early life ETS exposure in rats will induce measurable shifts in methylation at loci relevant to neurobehavioral outcomes that are detectable in both brain and blood. Whole genome bisulfite sequencing will identify ETS-vulnerable loci with validation by pyrosequencing. 2) Identify ETS-altered methylation-expression relationships in frontal cortex. We hypothesize that early life ETS exposure in rats induces changes in frontal cortex gene expression at ADHD relevant genes. Whole transcriptome profiling will identify these changes. We will also evaluate methylation expression relationships from the in vivo results and in an in vitro model of neuronal differentiation. 3) Determine if DNA methylation varies with ETS dose in humans. Prenatal and peri-natal cotinine levels and DNA methylation in children, from bisulfite pyrosequencing at ADHD-related genes and candidate loci from the above aims, will determine if methylation varies with exposure. The proposed research is expected to reveal ETS-related epigenetically vulnerable genes that mechanistically explain the link between ADHD and exposure to ETS and that may serve as biomarkers of past exposure.

Title: Disparities in cervical cancer precursors and deregulation of imprinted genes (Hoyo/Murphy, MPI)
Time Commitment: 20.0% 2.4 calendar
Supporting Agency: NIH/NCI 5R01-CA142983-04
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Andrea Bell, Grants Management Specialist, Email: bellan@mail.nih.gov, Phone: 301-496-3276, Fax: 301-496-8601
Performance Period:06/01/10 – 04/30/16
Level of Funding: $155,600
Goals: The purpose of the study is to examine if epigenetic deregulation of 12 imprinted genes is associated with progression to CIN2 or worse (CIN2+) in women with histologically confirmed CIN1 who are also infected with 'high risk' HPV.
Specific Aims: Aim 1: Evaluate aberrant methylation of imprinted genes, in exfoliated cervical cells, in relation to increased risk of progression to CIN2+ among 1,500 women with CIN1, and determine whether this association varies by race/ethnicity. Aim 2: Determine whether aberrant methylation of imprint regulatory elements in cervical cells is similar to that found in circulation, suggesting an early event; and if this is associated with (i) increased transcriptional expression, and (ii) loss of imprinting. Aim 3: Determine whether

deregulation of known imprinted genes in cervical cells can be used to discriminate women with CIN2+ among 500 ASCUS cases.

Title: Neurodevelopment and Improving Children's Health following EtS exposure (NICHES) Admin Core (Murphy)
Time Commitment: 6.2% 0.74 calendar
Supporting Agency: EPA (joint funding with NIH/NIEHS 1P01-ES022831-01)
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Alison Hanlon,Grants Specialist (202) 564-0244 Hanlon.Alison@epa.gov
Performance Period: 06/05/2013 – 05/31/2018
Level of Funding: $246,101
Goals: The Administrative Core will provide the infrastructure required to enable NICHES to effectively carryout its research objectives and translate scientific discoveries through education and clinical application. The Administrative Core will facilitate integration of the projects and community outreach efforts by leveraging unique resources at Duke in order improve the health of children and future generations.
Specific Aims: 1) Foster and maintain communication among team members, with the EAC, with the NIEHS and EPA, and with other Children's Centers; 2) Organize and integrate NICHES research, investigator training, and outreach activities to promote synergy and maximize the Center's impact on children's health in the community; 3) Promote the training of experts in cross-disciplinary fields in children's environmental health; 4) Manage fiscal and other resources; and 5) Track NICHES outputs and outcomes to ensure timely progress towards center goals.

Title: Epigenomic Consequences of Early Life Environmental Tobacco Smoke Exposure NICHES-Project 3 (Murphy)
Time Commitment: 10.6% 1.27 calendar
Supporting Agency: EPA (joint funding with NIH/NIEHS 1P01-ES022831-01)
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Alison Hanlon,Grants Specialist (202) 564-0244 Hanlon.Alison@epa.gov
Performance Period: 06/05/2013 – 05/31/2018
Level of Funding: $302,694
Goals: The results will for the first time mechanistically link tobacco smoke exposure in early life to neurobehavioral aspects of ADHD through epigenetics. The identified genes are highly likely to pave the way for development of novel diagnostic, prognostic and therapeutic tools that will ultimately improve children's health by allowing for earlier recognition of risk and intervention opportunities.
Specific Aims: 1) Identify ETS-related methylation targets. We will test the hypothesis that early life ETS exposure in rats will induce measurable shifts in methylation at loci relevant to neurobehavioral outcomes that are detectable in both brain and blood. Whole genome bisulfite sequencing will identify ETS-vulnerable loci with validation by pyrosequencing. 2) Identify ETS-altered methylation-expression relationships in frontal cortex. We hypothesize that early life ETS exposure in rats induces changes in frontal cortex gene expression at ADHD relevant genes. Whole transcriptome profiling will identify these changes. We will also evaluate methylation expression relationships from the in vivo results and in an in vitro model of neuronal differentiation. 3) Determine if DNA methylation varies with ETS dose in humans. Prenatal and peri-natal cotinine levels and DNA methylation in children, from bisulfite pyrosequencing at ADHD-related genes and candidate loci from the above aims, will determine if methylation varies with exposure. The proposed research is expected to reveal ETS-related epigenetically vulnerable genes that mechanistically explain the link between ADHD and exposure to ETS and that may serve as biomarkers of past exposure.

Title Validation and interrogation of differentially expressed and alternatively spliced genes in African American prostate cancer (Patierno)
Time Commitment: 3% 0.36 calendar

Supporting Agency: DoD W81XWH-14-0569 PC131972
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: CDMRP, 301-682-5507

Performance Period: 9/30/14-9/29/17
Level of Funding: $595,633
Goals: 1) To expand the sample cohort and delineate the relationship between the genetic/epigenetic/post-transcriptional factors in AA prostate cancer and Gleason grade.
2) To manipulate splicing using novel splice-switching oligonucleotides and determine functional outcomes.
Specific Aims: 1) Validate differentially expressed and spliced candidate genes in AA prostate cancer in an expanded sample cohort and define the relationship between these genes and Gleason grade. 2) Define the biologic significance of differences in cis-acting splicing elements of alternatively spliced candidate genes in AA prostate cancer to alternative splicing events specific to AA prostate cancer and define the relationship between these patterns and Gleason grade. 3) Use splice-switching oligonucleotides to delineate the biologic relevance of race-related differentially spliced genes involved in PIK3CD and MET signaling.

Title: Functional Genomic Screens of Tumor Recurrence in Ovarian Cancer (Chi)
Time Commitment: 5% 0.60 calendar
Supporting Agency: DoD W81XWH-14-0309 OC130493
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Karen Wylie, karen.m.wylie.ctr@mail.mil, 301-619-7016, Science Officer
Performance Period: 8/22/14-8/21/16
Level of Funding: $112,500
Goals: The goal is to apply functional genomic approaches to target the recurrent ovarian cancers.
Specific Aims: Aim 1: Functional genomic screen to identify kinases essential for recurrent ovarian cancers
Aim 2: Prioritize candidates and determine if inhibition of identified kinases will prevent recurrence

Title: Joint Environmental, Genetic and Epigenetic Regulation of Tyrosine Receptor Kinases and Childhood Respiratory Disease (Murphy – Duke site PI)
Time Commitment: 5% 0.6 calendar
Supporting Agency: University of Southern California/NIH R01-ES022216
Name and Address of the Funding Agency's Procuring Contracting/Grants Officer: Lillian Rivera, Contracts and Grants Officer, Email: lrivera@usc.edu, Phone: 323-442-2400
Performance Period: 9/13/13 –6/30/17
Level of Funding: $17,015
Goals: To test the hypothesize that epigenetic and genetic variation in tyrosine kinase signaling genes increases the susceptibility of children to the risk of childhood asthma and lower lung function when exposed to prenatal tobacco smoke.
Specific Aims: Specific aims are: (1) to investigate the association between PTS exposure and DNA methylation of TAM genes by (1a) measuring CpG methylation in selected CpG loci in 1252 newborn bloodspots from CHS children and (1b) validating the observed associations in cord blood from an independent sample of 600 subjects from Duke University's NEST cohort; (2) to investigate the association between PTS and DNA methylation of CpG loci in the promoters of mi-R34a and miR-199a/b levels, three miRNAs known to regulate AXL expression and (2a) to confirm that DNA methylation of mi-R34a and miR-199a/b promoters is associated with their expression levels and with expression of AXL using the NEST cohort; (3) to explore whether genetic variation in cis is associated with DNA methylation in the TAM genes and whether genetic variation modifies the effect of PTS on DNA methylation levels; and (4) to investigate whether PTS, epigenetic and/or genetic variation in the TAM genes are independently or jointly associated with childhood asthma and

with attained lung function at age 10 using novel hierarchical modeling methods to integrate the relative associations and contributions of each.

Title: Childrens Exposure to SVOC Mixtures Indoors and Associations with Obesity (Stapleton)
Time Commitment: 5% 0.60 calendar
Supporting Agency: NIH R01ES016099
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Grants Management Specialist(GMS) Molly Puente Email: puentem@mail.nih.gov Phone: 919-541-1371

Performance Period: 7/1/07-6/30/19
Level of Funding: $404,780
Goals: Obesity rates in the US, particularly in toddlers, are rapidly climbing and it has been hypothesized that early life exposure to chemicals which act as obesogens may be a contributing factor. Many contaminants which are ubiquitous in indoor dust are considered obesogens. The goal of this research is to measure pre- and postnatal exposures to mixtures of organic contaminants present in house dust, using both targeted and nontargeted (i.e. screening) approaches. We will also characterize the adipogenic potential of the house dust samples using a series of in vitro assays, and examine associations with obesity outcomes in toddlers. This work will contribute new data regarding the potential health impacts in children from chronic exposure to contaminants, and help identify mitigating factors.
Specific Aims: Aim 1: Characterize prenatal and postnatal exposure for a cohort of toddlers participating in the NEST study. A nested case control study will be conducted with 100 overweight/obese and 100 non-obese toddlers. Banked maternal plasma samples (1st trimester) will be analyzed for PFCs, OTs, and BPA as a prenatal exposure measure. Paired samples of serum, urine, handwipes and dust will be collected postnatally from the children (ages 2-3 yrs) born to these moms and analyzed using both targeted and newly-developed nontargeted approaches to identify multiple SVOC classes. Aim 2: Characterize potential PPARγ activity and adipogenic potential in indoor dust samples. Extracts from house dust will be characterized for competitive binding to PPARγ, and PPARγ agonism/antagonism using two high throughput in vitro assays. Dust samples will also be examined for adipogenic potential using 3T3-L1 cell culture assays. Effects directed analyses will then be conducted on the dust samples to identify the PPARγ active components in the dust mixtures. Aim 3: Examine the associations among obesity, pre- and postnatal exposure to individual chemicals and mixtures of chemicals, and PPARγ activity of the dust samples measured in the in vitro assays. We will use logistic regression to estimate associations of body mass index (BMI) with contaminant exposures (individual and combined using chemical mixtures models), and PPARγ activity/adipogenic potential of the dust samples.

OVERLAP

None

SPOC Reviewed: E. Brearley 10/28/15